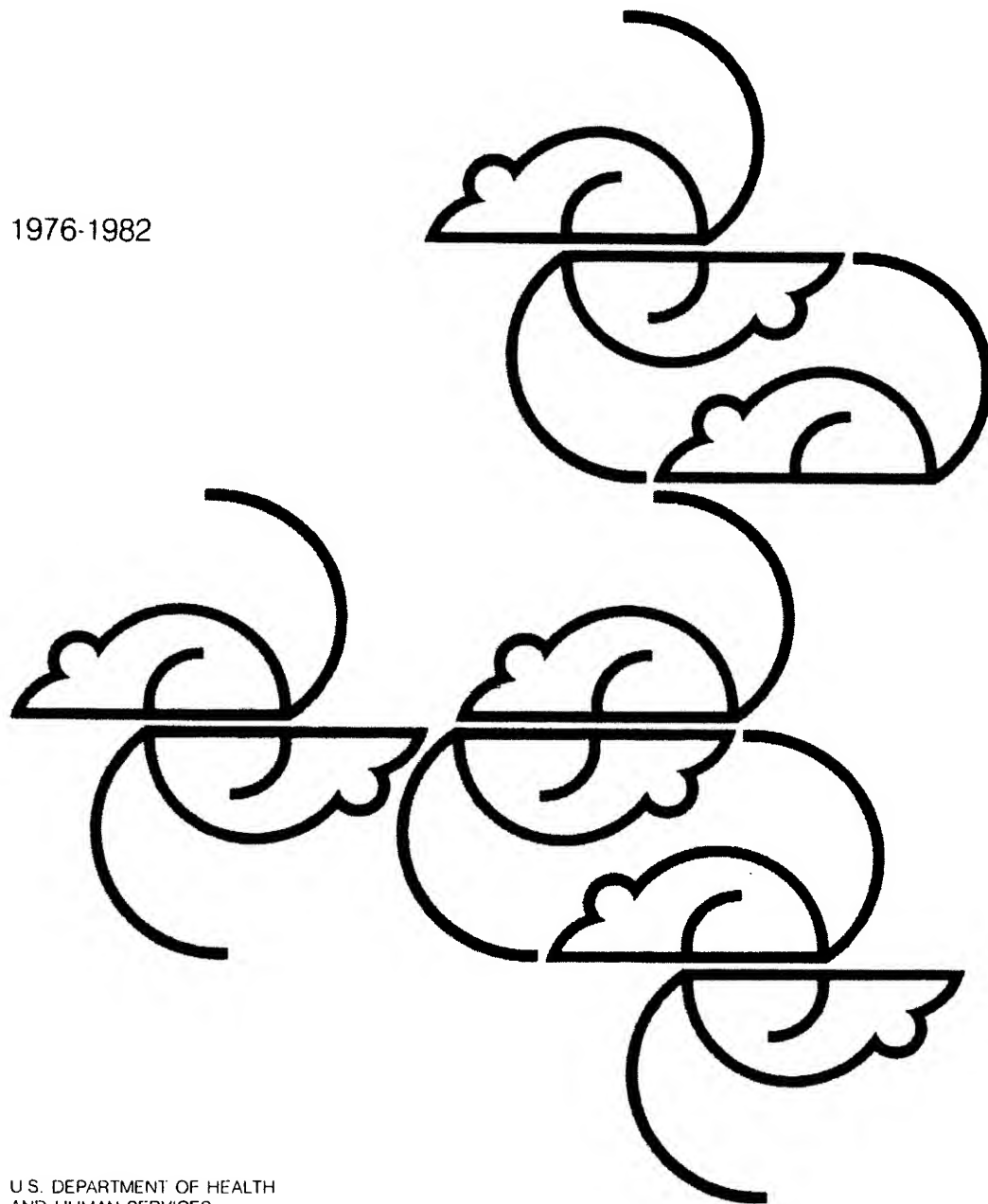

In Vivo Cancer Models

1976-1982



U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

In Vivo Cancer Models

1976-1982

Developmental Therapeutics Program
Division of Cancer Treatment
National Cancer Institute

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NIH Publication No. 84-2635
February 1984

This, the third edition of Screening Protocols, is dedicated to Dr. Ruth I. Geran, who retired on April 30, 1983, with thanks for her many contributions to its completion and to the Program. Dr. Geran served as Chairperson and/or member of the Protocol Committee of the Drug Evaluation Branch for much of her career with the National Cancer Institute.

Preface

The purpose of this publication is to supplement the existing protocols for screening new potential anticancer agents (Cancer Chemotherapy Reports, Part 3, Vol. 3, No. 2, September 1972) by providing information on the principal preclinical *in vivo* cancer models used during the period 1976-1982 by the Developmental Therapeutics Program (DTP) of the National Cancer Institute in the search for new agents for the treatment of cancer.

Appendix A lists the nine principal models used during this period. The reasons for the selection of these models were presented to the Division of Cancer Treatment (DCT) Board of Scientific Counselors at its meeting on November 10-11, 1975. Emphasis was to be placed during this period on the testing of agents against paired models of colon, breast, and lung tumors using human tumor tissue in parallel with mouse tumor tissue. The human tumor xenografts were to be implanted into athymic mice and the mouse tumor homografts were to be implanted into normal, immunocompetent mice. Lewis lung carcinoma was to be used, but alternative mouse lung tumor systems were to be investigated. Due to their demonstrated utility, leukemia L1210 and B16 melanoma were retained as part of the screening panel. The increased level of effort required to test a material in this enlarged tumor panel meant that the input of agents to this panel of cancer models would have to be limited in number. This raised the obvious question of the basis for selecting materials for broad spectrum screening. After considering a number of alternatives, a determination was made that the most reasonable approach would be an *in vivo* prescreen which had demonstrated sensitivity to most classes of clinically effective anticancer test agents but which was nevertheless sufficiently discriminating to limit the candidates adequately.

While the previous screen for synthetic agents (mouse leukemia L1210) was known to be highly predictive for clinical utility against human leukemias, lymphomas, and some solid tumors, the L1210 model was considered to be too restrictive for the new prescreen which was designed to minimize the chance of missing a test agent likely to show activity in one of the tumors in the enlarged panel. A comprehensive review of screening data on record indicated that mouse leukemia P388 would represent a more appropriate prescreen because (a) its response to test agents of various classes was qualitatively similar to that of L1210, and (b) the P388 model was quantitatively more sensitive than the L1210 model.

Despite the necessity of limiting the initial *in vivo* prescreen to one assay, it was recognized that no animal model was known to predict perfectly for clinical utility. Therefore, the input to the tumor panel was to be augmented with materials with antitumor activity reported in the world's literature and from other screening programs, and with test agents reported to exhibit relevant biochemical and other biological activities. Such agents were to be tested in the DCT prescreen to provide data for future analyses, and to be tested in the tumor panel regardless of prescreen results. In brief, agents which had shown interesting activity outside of the DCT would "bypass" the prescreen as a requirement for broad spectrum screening.

Appendix B summarizes the usual characteristics of the eight *in vivo* tumor panel models, the P388 leukemia prescreen, and other *in vivo* models of specific interest (e.g., transplanted mouse brain tumors) which were to be available to be utilized in specific instances. *In vitro* assays are to be the subject of a separate publication.

By design, materials that emerged as active from any of the panel models (or even very active in the P388 prescreen) were to be candidates for subjection to adequate clinical trial. This approach would prospectively provide critical information relative to the value of the preclinical testing with specific tumors or combinations of tumors.

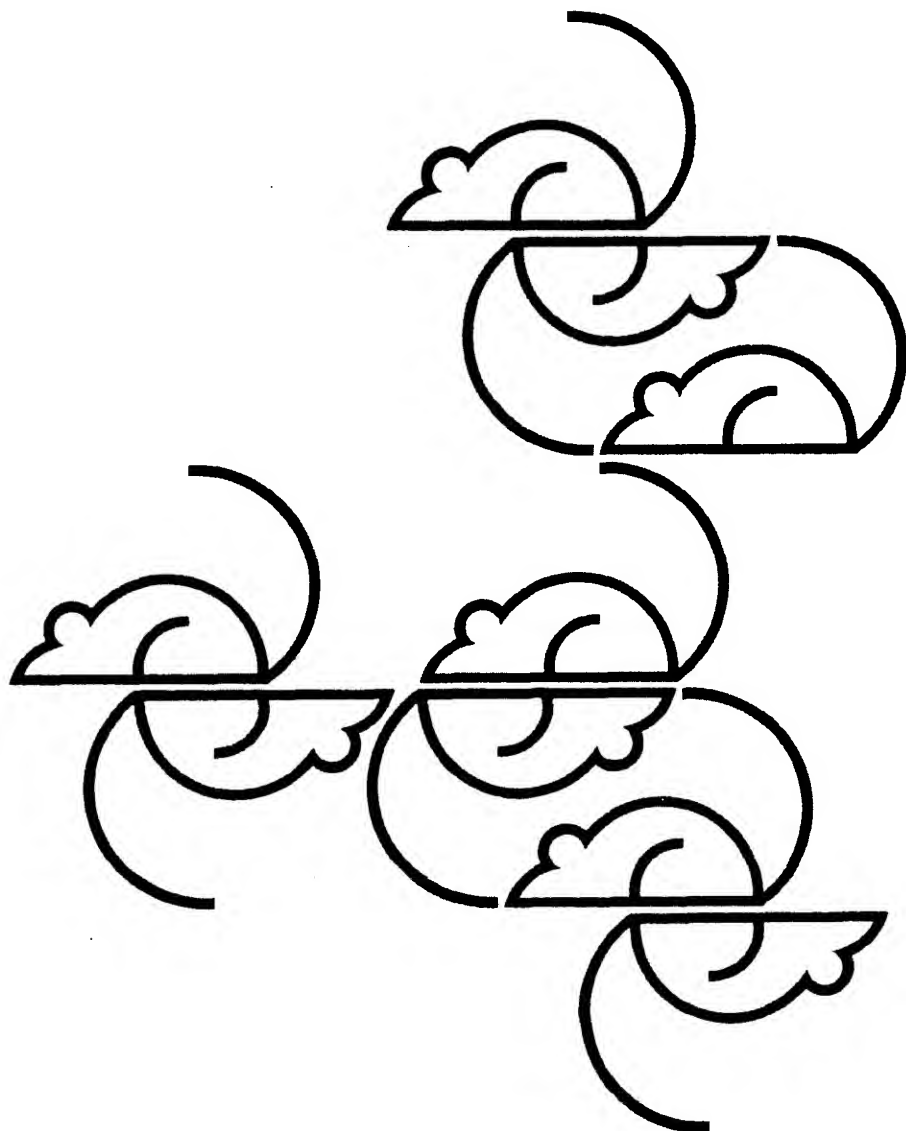
As the DCT panel of *in vivo* cancer models was phased into use, the assumption was that some preselection or priority rating (even for the prescreen) would have to be made. The logical basis for preselection would include demonstrated biological or biochemical data of some kind, and structural considerations.

Numbered protocols (e.g., "Refer to Protocol 8") cited in the following descriptions of individual models refer to "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems (Third Edition)" in Cancer Chemotherapy Reports, Part 3, Vol. 3, No. 2, September 1972.

Table of Contents

Preface	Page
	iii
Protocols for Selected <i>In Vivo</i> Models:	
3B131 Intraperitoneally-Implanted B16 Melanoma	2
3B132 Subcutaneously-Implanted B16 Melanoma	3
3B137 Intracranially-Implanted B16 Melanoma	4
3CDJ2 Subcutaneously-Implanted Staged Mammary Adenocarcinoma CD8F1	5
3CD72 Subcutaneously-Implanted Mammary Adenocarcinoma CD8F1	7
3C2G5 Subrenal Capsule Human Colon CX-1 Adenocarcinoma Xenograft	8
3C2H2 Subcutaneously-Implanted Human Colon CX-1 Adenocarcinoma Xenograft	10
3C631 Intraperitoneally-Implanted Colon 26 Adenocarcinoma	12
3C872 Subcutaneously-Implanted Colon 38 Carcinoma	13
3EM37/3EP37 Intracerebrally-Implanted Ependymoblastoma	14
3LE31/3LE21 Intraperitoneally-Implanted L1210 Leukemia	15
3LE37/3LE27 Intracranially-Implanted L1210 Leukemia	16
3LKG5 Subrenal Capsule Human Lung LX-1 Xenograft	17
3LKH2 Subcutaneously-Implanted Human Lung LX-1 Xenograft	19
3LL32 Subcutaneously-Implanted Lewis Lung Carcinoma	21
3LL39 Intravenously-Implanted Lewis Lung Carcinoma	22
3MBG5 Subrenal Capsule Human Mammary Carcinoma MX-1 Xenograft	23
3MBH2 Subcutaneously-Implanted Human Mammary Carcinoma MX-1 Xenograft	25
3PS31 Intraperitoneally-Implanted P388 Leukemia	27
3PS37 Intracranially-Implanted P388 Leukemia	28
Appendices:	
A List of the models (and their activity criteria) that comprise the <i>in vivo</i> prescreen and <i>in vivo</i> tumor panel	31
B List of characteristics of selected <i>in vivo</i> models	32

**Protocols for Selected
In Vivo Models**



(3B131) Intraperitoneally-Implanted B16 Melanoma

Origin of Tumor Line: Arose spontaneously in 1954 on the skin at the base of the ear in a C57BL/6 mouse.¹

Summary of Test Procedures: A 1:10 tumor brei is implanted i.p. in B₆C₃F₁ (or B₆D₂F₁) mice. I.p. test agent treatment begins one day after tumor implant and continues daily for a total of nine injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation: C57BL/6 mice.

Testing: B₆C₃F₁ (preferred) or B₆D₂F₁ mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Brei: Mix 1 gm of s.c. donor tumor and cold balanced salt solution equal to a final volume of 10 ml (for a 1:10 brei) and homogenize (preferred method).

Fragment: Prepare a 25 mg fragment of s.c. donor tumor.

Time: Day 10-14 (approximately Day 12).

Site: Implant s.c. into axillary region with puncture in inguinal region, using either 25 mg fragment or 0.5 ml of a 1:10 brei.

TESTING

Brei: Mix 1 gm of s.c. donor tumor and cold balanced salt solution equal to a final volume of 10 ml (for a 1:10 brei) and homogenize.

Time: Day 10-14 (approximately Day 12).

Site: Implant i.p. using 0.5 ml of a 1:10 brei.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every odd-numbered experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize and weigh animals. Treat as instructed.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Toxicity day for test animals and second animal weigh day. Prepare fresh test agent for subsequent testing.

Day 12: Control early-death day.

Day 30: Control no-take day.

Day 60: End and evaluate experiment.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 119875* at doses of 2 and 1 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. QD 1-9. The lower T/C limit for the positive control is 135%. The acceptable untreated control median survival time is 15-22 days.

Evaluation: (refer to Protocol 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with > 65% survivors on Day 5. A T/C value of < 86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

A T/C \geq 125% is considered necessary to demonstrate moderate activity. A reproducible T/C value \geq 150% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹Handbook on Genetically Standardized Jax Mice. Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, 1962. See also Ann NY Acad Sci, vol 100, Parts 1 and 2 (Conference on the Biology of Normal and Typical Pigment Cell Growth of 1961), 1963.

*Positive control compound NSC 119875 is Cisplatin (USAN). CAS RN is 15663-27-1.

(3B132) Subcutaneously-Implanted B16 Melanoma

Origin of Tumor Line: Arose spontaneously in 1954 on the skin at the base of the ear in a C57BL/6 mouse.¹

Summary of Test Procedures: Either a 1:10 tumor brei or 25 mg fragment is implanted s.c. in B₆D₂F₁ mice. I.p. test agent treatment begins one day after tumor implant and continues daily for a total of nine injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation: C57BL/6 mice.

Testing: B₆D₂F₁ mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: Once source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)

PROPAGATION

Brei: Mix 1 gm of s.c. donor tumor and cold balanced salt solution equal to a final volume of 10 ml (for a 1:10 brei) and homogenize (preferred method)

Fragment: Prepare a 25 mg fragment of s.c. donor tumor.

Time: Day 10-14 (approximately Day 12).

Site: Implant s.c. into axillary region with puncture in inguinal region, using either 25 mg fragment or 0.5 ml of a 1:10 brei.

TESTING

Brei: Mix 1 gm of s.c. donor tumor and cold balanced salt solution equal to a final volume of 10 ml (for a 1:10 brei) and homogenize (preferred method)

Fragment: Prepare a 25 mg fragment of s.c. donor tumor.

Time: Day 10-14 (approximately Day 12).

Site: Implant s.c. into axillary region with puncture in inguinal region, using either 25 mg fragment or 0.5 ml of a 1:10 brei.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every odd-numbered experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize and weigh animals. Treat as instructed.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Toxicity day for test animals and second animal weigh day. Prepare fresh test agent for subsequent testing.

Day 13: Control early-death day.

Day 46: Control no-take day.

Day 60: End and evaluate experiment.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 19893* at 20 mg/kg/injection, or NSC 26271* at 50 mg/kg/injection or NSC 125066* at 12, 6, and 3 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. QD 1-9. The lower T/C limit for the positive control is 135%. The acceptable untreated control median survival time is 21-31 days.

Evaluation: (refer to Protocols 4 and 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with > 65% survivors on Day 5. A T/C value of < 86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

A T/C \geq 140% is considered necessary to demonstrate moderate activity. A reproducible T/C \geq 150% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹Handbook on Genetically Standardized Jax Mice. Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, 1962. See also Ann NY Acad Sci, vol 100, Parts 1 and 2 (Conference on the Biology of Normal and Typical Pigment Cell Growth of 1961), 1963

*Positive control compound NSC 19893 is 5-FU CAS RN is 51-21-8
Positive control compound NSC 26271 is Cytosin CAS RN is 50-18-0
Positive control compound NSC 125066 is Bleomycin CAS RN is 110506-07

(3B137) Intracranially-Implanted B16 Melanoma

Origin of Tumor Line: Arose spontaneously in 1954 on the skin at the base of the ear in a C57BL/6 mouse.¹

Summary of Test Procedures: 1×10^5 cells in suspension are implanted i.c. for testing in B₆D₂F₁ mice. I.p. test agent treatment begins one day after implant or is delayed per instructions. The test agent is administered i.p. daily for a total of nine injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation: C57BL/6 mice.

Testing: B₆D₂F₁ mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Brei: Mix 1 gm of s.c. donor tumor and cold balanced salt solution equal to a final volume of 10 ml (for a 1:10 brei) and homogenize (preferred method).

Fragment: Prepare a 25 mg fragment of s.c. donor tumor.

Time: Day 10-14 (approximately Day 12).

Site: Implant s.c. into axillary region with puncture in inguinal region, using either 25 mg fragment or 0.5 ml of a 1:10 brei.

TESTING

Suspension: Prepare 0.05 ml of tumor cell suspension containing 1×10^5 cells.

Time: Day 10-14 (approximately Day 12).

Site: Implant i.c. using 0.05 ml of 1×10^5 intact cells suspended in cold balanced salt solution.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every odd-numbered experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day of First Injection (usually Day 1): Randomize and weigh animals (refer to Protocol 10). Treat as instructed, with injection volume based on individual body weight for that day.

Day of Initial Toxicity Evaluation: Four days after first treatment. On this day, final animal body weights are also to be recorded.

Day 7: Control early-death day.

Day 24: Control no-take day (approximate midpoint between last acceptable day of control median survival time and day of final evaluation). Refer to Protocol 11.201.

Day 30: End and evaluate experiment. If any test other than the positive control compound has more than half of its animals surviving on that day, postpone Final Evaluation Day until Day 60.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 409962* at 32 and 24 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. Day 1 only. The lower T/C limit for the positive control is 140%. The acceptable untreated control median survival time is 11-18 days.

Evaluation: (refer to Protocol 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with $> 65\%$ survivors on Day 5. A T/C value of $< 86\%$ indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

A T/C $\geq 125\%$ is considered necessary to demonstrate moderate activity. A reproducible T/C of $\geq 150\%$ is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹Handbook on Genetically Standardized Jax Mice, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, 1962. See also Ann NY Acad Sci, vol 100, Parts 1 and 2 (Conference on the Biology of Normal and Typical Pigment Cell Growth of 1961), 1963.

*Positive control compound NSC 409962 is BCNU CAS RN is 154-93-8

(3CDJ2) Subcutaneously-Implanted Staged Mammary Adenocarcinoma CD8F1

Origin of Tumor Line: Spontaneous tumors, predominantly papillary adenocarcinomas, arising in CD8F1 female mice are used for first generation transplants. A CD8F1 mouse is the product of a cross between a female of an inbred virus-infected colony of BALB/cCMC¹ and a male of an inbred colony of DBA/8-CMC.²

Summary of Test Procedures: A 1:20 tumor brei is prepared from a minimum of four spontaneous tumors and 0.3 ml is implanted s.c. in the axillary region in CD8F1 mice. The test agent is administered i.p. once on Staging Day. The parameter is change in tumor weight.

Animals: (refer to Protocol 8)

Propagation: Arise spontaneously in CD8F1 females.

Testing: CD8F1 mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2 and 6)

PROPAGATION

Time: } Tumors arise spontaneously.
Site: }

TESTING

Tissue:

Brei: Prepare a 1:20 tumor brei. Weigh non-necrotic tumor tissue from at least 4 spontaneous tumors. Mince and press through 40 mesh screen using cold balanced salt solution. Prior to use, pour through a second 40 mesh screen to remove larger fragments. Final brei is 1 gm of tumor material and cold balanced salt solution equal to a final volume of 20 ml.

Time: Tumors arise spontaneously.

Site: Implant s.c. 0.3 ml of a 1:20 tumor brei in axillary region with puncture in inguinal region.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7).

Day 1: Check cultures. Discard experiment if contaminated.

Day 2: Recheck cultures. Discard experiment if contaminated.

Staging Day and Treatment Day: Select mice with tumors weighing no less than 100 mg nor more than 700 mg. Prepare materials. Test positive control compound in every odd-numbered experiment. Randomize and treat by individual body weight. Inject test agent i.p. on Staging Day only. Record total group body weight (Weigh Day 1). Record deaths daily.

Toxicity Day: Seven days post Staging Day (same as Final Evaluation Day).

Final Evaluation Day: Seven days post Staging Day. End and evaluate experiment. Record total group animal weights (Weigh Day 2), measure tumors with calipers (refer to Protocol 11.301 and EVALUATION below) and record.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 26271* at a dose of 250 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. Staging Day only. The T/C limit for the positive control is $\leq 20\%$. The acceptable untreated control median tumor weight change range is 400-2000 mg.

Evaluation: (refer to Protocol 11)

The parameter measured is median tumor weight change based on length and width measurements in millimeters. Compute mean animal body weights for Staging Day and Final Evaluation Day, compute T/C for all test groups with $> 65\%$ survivors on Final Evaluation Day. An excessive animal body weight change difference (test minus control) may also be used in evaluating toxicity.

On Staging Day and on Final Evaluation Day, measure and record length and width measurements for tumors of individual mice, measuring with calipers in millimeters.

1. Calculate tumor weights (mgs) from tumor dimensions (mm \times mm) following the formula for volume of a prolate ellipsoid:

$$\frac{L \times W^2}{2} \quad \text{Where L is the longer of the two measurements, and the first value recorded.}$$

2. Calculate the change in tumor weight (delta) for each group by subtracting the group median tumor weight on Staging Day from group median tumor weight on Final Evaluation Day:

$$\Delta Wt = W_{\text{FINAL}} - W_{\text{INITIAL}}$$

3. Calculate median tumor weight change (ΔWt) for test (T) and control (C) groups.

¹CMC—Catholic Medical Center of Brooklyn and Queens Inc
²ibid.

*Positive control compound NSC 26271 is Cytosar CAS RN is 50-18-0

4. Calculate T/C% for all test groups with > 65% survivors on Final Evaluation Day:

$$T/C\% = \frac{\Delta WtT}{\Delta WtC} \times 100 \text{—if } \Delta WtT \text{ positive.}$$

$$T/C\% = \frac{\Delta WtT}{\text{Test Mean Tumor Weight}_{\text{INITIAL}}} \times 100 \text{—if } \Delta WtT \text{ negative}$$

Criteria for Activity:

A $T/C \leq 20\%$ is considered necessary to demonstrate moderate activity. A reproducible $T/C \leq 0\%$ is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

(3CD72) Subcutaneously-Implanted Mammary Adenocarcinoma CD8F1

Origin of Tumor Line: Spontaneous tumors, predominantly papillary adenocarcinomas, arising in CD8F1 female mice are used for first generation transplants. A CD8F1 mouse is the product of a cross between a female of an inbred virus-infected colony of BALB/c-CMC¹ and a male of an inbred colony of DBA/8-CMC.²

Summary of Test Procedures: A 1:20 tumor brei is prepared from a minimum of 4 spontaneous tumors and 0.3 ml is implanted s.c. in the axillary region in CD8F1 mice. I.p. test agent treatment starts one day after tumor implant and is repeated every seventh day for a total of five injections. The parameter is median tumor weight. Results are expressed as a percentage of control tumor weight.

Animals: (refer to Protocol 8)

Propagation: Arise spontaneously in CD8F1 females.

Testing: CD8F1 mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2 and 6)

PROPAGATION

Time: } Tumors arise spontaneously.
Site: }

TESTING

Brei: Prepare a 1:20 tumor brei. Weigh non-necrotic tumor tissue from at least 4 spontaneous tumors. Mince and press through 40 mesh screen using cold balanced salt solution. Prior to use, pour through a second 40 mesh screen to remove larger fragments. Final brei is 1 gm of tumor and cold balanced salt solution equal to a final volume of 20 ml.

Time: Tumors arise spontaneously.

Site: Implant s.c. 0.3 ml of a 1:20 tumor brei in axillary region with puncture in inguinal region.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every odd-numbered experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize, weigh, and record weights of all mice. Begin treatment Day 1 and treat Q7D \times 5. Inject all mice on basis of individual body weight.

Day 2: Recheck cultures. Discard experiment if contaminated.

Days 8, 15, 22, 29: Prepare test agent fresh for each injection day. Weigh mice (but do not record) and inject according to individual body weight.

Day 30: Toxicity and Final Evaluation Day. Weigh animals and record. Measure tumors with calipers and calculate tumor weights (refer to Protocol 11.301). End and evaluate experiment.

Quality Control: (Protocol 7)

Schedule the positive control compound (NSC 26271* at a dose of 37.5 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. Q7D \times 5 beginning on Day 1. The T/C limit for the positive control is \leq 42%. The acceptable untreated control final median tumor weight is 300 - 5000 mg.

Evaluation: (Protocol 11)

The parameter measured is median tumor weight based on length and width measurements in millimeters. Compute mean animal body weights for Day 1 and Day 30, compute T/C for all test groups with $>$ 65% survivors on Day 30. An excessive animal body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

A initial T/C \leq 42% is considered necessary to demonstrate moderate activity. A reproducible T/C \leq 10% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a test status code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹CMC-- Catholic Medical Center of Brooklyn and Queens Inc
2000

*Positive control compound NSC 26271 is Cytosin CAS RN is 50 18 0

(3C2G5) Subrenal Capsule Human Colon CX-1 Adenocarcinoma Xenograft

Origin of Tumor Line: Surgical explant from the primary colon tumor of a 44 year old woman with no previous chemotherapy was established in tissue culture. This *in vitro* tumor line was transferred to athymic mice. Carried in athymic mice, it is a moderately well differentiated adenocarcinoma consistent with GI origin. (Reference: Tumor Bank information.)

Summary of Test Procedures: Tumor fragment implanted under the membranous covering of the kidney of either athymic Swiss or athymic random bred mice. S.c.* test agent treatment starts one day after tumor implant and is repeated every fourth day for a total of four injections. The parameter is change in tumor weight.

Animals: (refer to Protocol 8)

Propagation and Testing: Athymic Swiss (Cr:NIH(S)-nu) or athymic random bred (NCr-nu) mice.

Weight: Mice should be within a 4 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Age: Record age of mice.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size:

General Testing: Six animals per test group and 12 per control. Typically, a single test agent experiment is run with 4 dose levels of the test agent at 50% intervals, using 6 mice per test group and 12 mice per vehicle control group. Total number of mice in a typical single test agent experiment (tests and control) is 36.

Tumor Transfer: (refer to Protocols 2, 5, and 6)

PROPAGATION

Fragment: Prepare a 2×2×2 mm fragment of s.c. donor tumor.

Time: When donor tumor reaches 500-800 mg (approximately 21 days after implant).

Site: Implant fragment s.c. into axillary region with puncture in inguinal region using a 13 gauge trocar.

TESTING

Fragment: Prepare a 10×10×10 Ocular Micrometer Unit (OMU) fragment. Average diameter must be 9-12 OMU's measured under a dissecting microscope. 10 OMU's = 1 mm.

Anesthesia: Any satisfactory anesthesia (e.g., chloral hydrate, Avertin, etc.).

Medium: Tissue culture medium with no antibiotics (e.g., 199, Eagles MEM, or Earles).

Time: When donor tumor reaches 500-800 mg (approximately 21 days after implant).

Site: Implant fragment under the subrenal capsule, using a 16 gauge trocar with a 22° bevel, after exposing the kidney with a 7 mm dorsal skin incision. The wound is closed with a 9 mm wound clip after closing the peritoneum with 1-4 silk sutures.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Anesthetize animals. Record body weight (Weigh Day 1). Implant tumor, measure, and record. Randomize animals after they recover from the anesthetic. Run bacterial cultures (refer to Protocol 7). Determine solubilities of test agents. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Prepare materials. Initiate s.c.* test agent injections (in the nape of the neck) based upon individual body weight. Treatment is Q4D on Days 1, 5, 9, and 13. Prepare test agent fresh on each injection day and administer based on individual body weight for that day.

Day 2: Recheck cultures. Discard experiment if contaminated.

Days 5, 9 and 13: Prepare test agent fresh on each injection day and administer based on individual body weight for that day.

Day 15: End and evaluate experiment. Record body weights (Weigh Day 2). Measure tumor in OMU's. Final Evaluation Day for this model is also test toxicity day, test no-take day, control early-death day, control no-take day, and Weigh Day 2.

Quality Control:

- 1) Quality control of this tumor line is the responsibility of the Tumor Bank. Tumors not properly sensitive to a positive control test agent will not be supplied.
- 2) Implant 2 or 3 additional mice which can be used for replacement for surgical deaths. If surgical deaths do not occur, use these mice as additional control animals.
- 3) Within a given experiment, whenever possible use mice from the same supplier, date of receipt, and shipping crate to reduce fighting. If mice fight, house fighters individually.
- 4) House mice 3 to 6 per cage.
- 5) Donor tumor should weigh between 500-800 mg and be scrupulously cleaned of necrotic and/or hemorrhagic areas.
- 6) In case of unusual deaths, these animals should be autopsied and peculiarities noted.
- 7) Specific definitions for subrenal capsule implants for Control Status Code assignments by the computer (refer to Protocol 7.7) are:
 - a. Acceptable control mean tumor weight change is $\geq 20\%$ between Day 0 and Final Evaluation Day.
 - b. Control no-take: A mouse with a tumor weight increase of $< 20\%$ between Day 0 and Final Evaluation Day. (Computer determined.)
 - c. Excessive control no-takes: 2 or more no-takes are excessive in a control group of 12 mice (refer to Protocol 7.3).
 - d. Excessive control early deaths: 2 or more control deaths in a group of 12 to 19 animals (i.e., $\geq 10\%$) on or before Final Evaluation Day.

*Changed to s.p. in October 1982

Evaluation:

The parameter measured is mean tumor weight change (delta) based on length and width measurements in millimeters. Compute mean animal body weights for Day 1 and Day 11, compute T/C for all test groups with > 65% survivors on Day 11. An excessive animal body weight change difference (test minus control) may also be used in evaluating toxicity.

The NCI screening laboratories on Day 0 and on Final Evaluation Day are to measure and input OMU length and width measurements for tumors. The dimensions are measured and recorded in OMU's. (They will be entered on the WS 180 Solid Tumor Data Form using type 2 with code H, per instructions of 9/81 from the Screener Instructions for Use of the Solid Tumor Input Form, section 3.3.1.2. By convention, the length (L) dimension must be entered first.) The NCI computer:

1. Converts OMU's to millimeters (mm).
2. Calculates tumor weights (mgs) from tumor dimensions (mm × mm) following the formula for the volume of a prolate ellipsoid:

$$\frac{L \times W^2}{2} \quad \text{Where L is the longer of the two measurements.}$$

3. Calculates the change (delta) in mean tumor weight for each group of mice:

$$\text{Change in Mean Tumor Weight} = \text{Mean Tumor Weight}_{\text{FINAL}} - \text{Mean Tumor Weight}_{\text{INITIAL}}$$

4. Calculates the change (delta) in mean tumor weight for test (T) and control (C) groups.
5. Calculates T/C% for all test groups with > 65% survivors on Final Evaluation Day:

$$T/C\% = \frac{\Delta WtT}{\Delta WtC} \times 100 \text{—if } \Delta WtT \text{ positive.}$$

$$T/C\% = \frac{\Delta WtT}{\text{Test Mean Tumor Weight}_{\text{INITIAL}}} \times 100 \text{—if } \Delta WtT \text{ negative.}$$

Criteria for Activity:

An initial T/C ≤ 20% is considered necessary to demonstrate moderate activity. A reproducible T/C ≤ 10% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports. Input data. Screener assigns a code of "U" to an individual mouse whose response screener considers invalid, including the following circumstances:

- 1) Tumor lost from site of implant and kidney appears normal.
- 2) Animal dies or appears ill and loses weight—not attributable to test agent toxicity—for any reason, including fighting.
- 3) More than 1 tumor present.
- 4) Infection at site of implant.
- 5) Kidney does not appear normal.

A comment must accompany all "U" code designations.

The screener designates as unsatisfactory (assigns a Test Status Code of 33) all test groups that the screener considers invalid for any reason, including a case where more than 33% of the mice have been assigned a "U" code. The computer designates as unsatisfactory (assigns a Test Status Code of 34) all tests where:

- 1) There is no control delta calculated.
- 2) More than 33% of test mice have been assigned a "U" code.
- 3) Less than 67% of "Non-U" test mice are acceptable for calculation (i.e., initial tumor diameters are between 9 to 12 OMU's and final measurements exist for Final Evaluation Day).
- 4) Test groups where the control group contains 10% or more spontaneous tumor regressions.

The computer assigns the appropriate Control Status Code to reflect the acceptability of the control group, using definitions listed under "7" of Quality Control in addition to the general definitions of Protocol 7.7 and Instruction 14.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

(3C2H2) Subcutaneously-Implanted Human Colon CX-1 Adenocarcinoma Xenograft

Origin of Tumor Line: Surgical explant from the primary colon tumor of a 44 year old woman with no previous chemotherapy was established in tissue culture. This *in vitro* tumor line was transferred to athymic mice. Carried in athymic mice, it is a moderately well differentiated adenocarcinoma consistent with GI origin. (Reference: Tumor Bank information.)

Summary of Test Procedures: Tumor fragment implanted subcutaneously in the axillary region of either athymic Swiss or athymic random bred mice. I.p. test agent treatment starts when tumors reach a weight range of 100-700 mg and is repeated every fourth day for a total of three injections. The parameter is change in tumor weight.

Animals: (refer to Protocol 8)

Propagation and Testing: Athymic Swiss (Cr:NIH(S)-nu) or athymic random bred (NCR-nu) mice.

Weight: Mice should be within a 4 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Age: Record age of mice.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Six to 10 earmarked animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)

PROPAGATION

Fragment: Prepare a 25 mg fragment of s.c. donor tumor.

Time: Day 35 (approximately).

Site: Implant fragment s.c. into the axillary region with a puncture in the inguinal region.

TESTING

Fragment: Prepare a 14 mg fragment of s.c. donor tumor.

Time: Day 35 (approximately).

Site: Implant fragment s.c. into the axillary region with a puncture in the inguinal region.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7).

Day 1: Check cultures. Discard experiment if contaminated. Record deaths daily.

Day 2: Recheck cultures. Discard experiment if contaminated.

Staging Day (Initial Treatment Day): Select mice with tumors weighing no less than 100 mg and no more than 700 mg.

Prepare materials. Randomize and treat by individual body weight. Inject test agent i.p. on Staging Day and continue every 4 days for a total of 3 injections. Record total group body weight (Weigh Day 1).

Measurement Days: Body weights and tumor measurements are recorded on Initial Treatment Day (Staging Day) and selected measurement days.

Final Evaluation Day: Variable—that measurement day which yields the optimum (best) T/C% is designated Final Evaluation Day. End and evaluate experiment.

Quality Control: (refer to Protocol 7)

Not established.

Evaluation: (refer to Protocol 11)

The parameter measured is mean tumor weight change (delta) based on length and width measurements in millimeters.

Tumors are considered eligible for evaluation in the period 7 to 21 days post initial treatment for single injection treatment and 12 to 21 days post initial treatment for all other regimens. Measurement days should be selected such that the entire evaluation eligibility period is examined (i.e., post initial treatment days 13, 17, and 21 as an example). T/C% is calculated for only those groups where the survivors on the final possible evaluation day are greater than 65%. An excessive animal body weight change difference (test minus control) may also be used in evaluating toxicity.

Parameter: Calculations are based on group weights. Animals are ear-marked only to permit total elimination of data for any animal designated as a no-take, any animal that escaped, or any animal that for any reason is deemed by the screener to be unacceptable for inclusion in the calculations.

Mean tumor weights are calculated for each measurement day within the eligibility period as each day is potentially a Final Evaluation Day. For each such day, change (delta) in mean tumor weight is calculated for both the test and control, as follows:

1) Calculates the change (delta) in mean tumor weight for test (T) and control (C) groups.

$$\text{Change (delta) in Mean Tumor Weight} = \text{Mean Tumor Weight}_{\text{FINAL}} - \text{Mean Tumor Weight}_{\text{INITIAL}}$$

2) Calculate T/C% for all test groups with greater than 65% survivors on post staging Day 21.

$$T/C\% = \frac{\Delta WtT}{\Delta WtC} \times 100 \text{—if } \Delta WtT \text{ positive.}$$

$$T/C\% = \frac{\Delta WtT}{\text{Test Mean Tumor Weight}_{\text{INITIAL}}} \times 100 \text{—if } \Delta WtT \text{ negative.}$$

Determine optimum T/C% and the day of its occurrence to establish the final T/C% evaluation and Final Evaluation Day.

Criteria for Activity:

An initial T/C \leq 20% is considered necessary to demonstrate moderate activity. A reproducible T/C \leq 10% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

(3C631) Intraperitoneally-Implanted Colon 26 Adenocarcinoma

Origin of Tumor Line: Chemically induced in the colon of a C57BL/6 mouse in 1973 with repeated intrarectal instillation of N-methyl-N-nitrosourea.¹

Summary of Test Procedures: A 1:100 tumor brei is implanted i.p. in CD₂F₁ mice. I.p. test agent treatment starts one day after tumor implant and is repeated on Day 5 for a total of two injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation: BALB/cJ mice.

Testing: CD₂F₁ mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: A minimum of 30 control animals must be used, otherwise, the number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Fragment: Prepare a 100 mg fragment from 400-800 mg fragment of s.c. donor tumor without surface ulceration.

Time: When donor tumor reaches 400-800 mg (approximately Day 12-15).

Site: Implant 100 mg fragment s.c. into the axillary region with a puncture in the inguinal region.

TESTING

Brei: Pool donor tumors (400 to 1000 mg nonulcerated) in cold balanced salt solution. Mince into 5-10 mg fragments. Draw fragments in and out of 5 cubic centimeter glass syringe—without needle—until pieces are broken into smaller fragments. Pour brei onto 40-mesh sieve over beaker, scraping brei with forcep points until all the liquid is passed through the mesh. Pour brei into graduated 15 ml centrifuge tubes, and centrifuge for 5 minutes at approximately 2,500 RPM (in IEC clinical centrifuge use maximum speed). Pour off supernatant. Estimate number of milliliters of cells in each tube by using a point one half way up slope of cells. Multiply this number by 99, which gives the volume of cold balanced salt solution needed to give a 1 percent solution. Aspirate cells into and out of a pipette using a rubber bulb. Transfer into sterile bottles and cap.

Time: When donor tumor reaches 400-1000 mg (approximately Day 12-15).

Site: Implant i.p. using 0.5 ml of brei.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in odd-numbered experiments. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize and weigh animals (refer to Protocol 10).

Days 1 and 5: Unless an alternate or additional schedule is specified, administer test agents i.p. on Days 1 and 5. (All treatments are based on average animal body weight on day of treatment.)

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Second weigh day and toxicity day.

Day 8: Control early-death day.

Day 60: Control no-take day. End and evaluate experiment.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 95441* suspended in Klucel at a dose of 10 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. Q4D × 2 beginning on Day 1. The lower T/C limit for the positive control is 150%. The acceptable untreated control median survival time is 16-29 days.

Evaluation: (refer to Protocol 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with > 65% survivors on Day 5. A T/C value of < 86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

An initial T/C ≥ 130% is considered necessary to demonstrate moderate activity. A reproducible T/C ≥ 150% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹"Tumor Induction Relationships in Development of Transplantable Cancers of the Colon in Mice for Chemotherapy Assays with a Note on Carcinogen Structure," *Cancer Research* 35, pp. 2434-2439, 1975

*Positive control compound NSC 95441 is trans-Methyl-CCNU CAS RN is 33073-59-5

(3C872) Subcutaneously-Implanted Colon 38 Carcinoma

Origin of Tumor Line: Chemically induced in the colon of a C57BL/6 mouse in 1973 with repeated s.c. injections of 1, 2-dimethylhydrazine.¹

Summary of Test Procedures: A tumor fragment is implanted s.c. i.p. test agent treatment starts two days after tumor implant and is repeated on Day 9 for a total of two injections. The parameter is median tumor weight. Results are expressed as a percentage of the control tumor weight.

Animals: (refer to Protocol 8)

Propagation: C57BL/6 mice.

Testing: B₆C₃F₁ or B₆D₂F₁ mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: A minimum of 30 control animals must be used, otherwise, the number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Fragment: Prepare a 70 mg (acceptable range 60-80 mg) fragment from 400-2000 mg s.c. donor tumor without surface ulceration.

Time: When donor tumor reaches 400-2000 mg (approximately Day 18-25).

Site: Implant 70 mg fragment s.c. into the axillary region with a puncture in the inguinal region.

TESTING:

Fragment: Prepare a 70 mg (acceptable range 60-80 mg) fragment from 400-2000 mg s.c. donor tumor without surface ulceration. For testing, this must be a single fragment.

Time: When donor tumor reaches 400-2000 mg (approximately Day 18-25).

Site: Implant 70 mg fragment s.c. into the axillary region with a puncture in the inguinal region.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Test positive control compound in odd-numbered experiments.

Day 1: Check cultures. Discard experiment if contaminated.

Day 2: Recheck cultures. Discard experiment if contaminated. Randomize animals. Record individual and total animal weights. Prepare materials.

Days 2 and 9: Administer test agent i.p. based on the average animal body weight on day of treatment.

Day 20: End and evaluate experiment. Record individual and total mouse weights (including tumor weights). Record survivors for toxicity day. Determine individual tumor weights by caliper measurements (refer to Protocol 11.301 and EVALUATION below). Calculate and record median tumor weight for group. Individual tumor weights should be reported.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 19893* at a dose of 70 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. Q7D x 2 beginning on Day 2. The upper T/C limit for the positive control is $\leq 42\%$. The acceptable untreated control final median tumor weight is 400-2000 mg.

Evaluation: (refer to Protocol 11)

The parameter measured is median tumor weight based on length and width measurements in millimeters. Compute mean animal body weights for Day 2 and Day 20, compute T/C for all test groups with $> 65\%$ survivors on Day 20.

Criteria for Activity:

An initial T/C $\leq 42\%$ is considered necessary to demonstrate moderate activity. A reproducible T/C $\leq 10\%$ is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹"Tumor Induction Relationships in Development of Transplantable Cancers of the Colon in Mice for Chemotherapy Assays with a Note on Carcinogen Structure," *Cancer Research* 35, pp 2434-2439, 1975

*Positive control compound NSC 19893 is 5-FU. CAS RN is 51 21-8

(3EM37 or 3EP37) Intracerebrally-Implanted Ependymoblastoma

Origin of Tumor Line: Chemically induced in the brain of a C₃H male mouse in 1941 by intracranial implantation of methylcholanthrene. The mutant subline used by DCT was derived over twenty years later and has a more rapid growth rate.¹

Summary of Test Procedures: A tumor fragment is implanted intracerebrally in B₆C₃F₁ (or C57BL/6) mice. I.p. test agent treatment starts one day after tumor implant and continues daily for a total of five injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation: C57BL/6 mice.

Testing: B₆C₃F₁ (preferred), or C57BL/6 mice.

Weight: Mice must weigh between 20 and 24 grams.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Fragment: Prepare a 2×2×8 mm fragment of s.c. donor tumor using a 13 gauge trocar.

Time: Day 14.

Site: Implant s.c. into axillary region with puncture in inguinal region using a 13 gauge trocar.

TESTING

Fragment: Prepare a 1×1×1 mm fragment of s.c. donor tumor.

Suspension: Suspend 1×10⁵ cells in 0.05 ml of cold balanced salt solution.

Time: Day 14.

Site: Implant fragment intracerebrally using a 19 gauge spinal needle with special guard, or implant suspension intracerebrally using a 26 gauge needle.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials for early treatment. Test positive control compound in every odd-numbered experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize and weigh animals. Administer test agent (based on individual body weight for that day).

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Test toxicity day and second animal weigh day.

Day 10: Control early-death day.

Day 40: Control no-take day.

Day 60: End and evaluate experiment.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 409962* at doses of 8, 4, and 2 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. QD1-5. The lower T/C limit for the positive control is 135%. The acceptable untreated control median survival time is 14-30 days.

Evaluation: (refer to Protocol 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with > 65% survivors on Day 5. A T/C value < 86% indicates toxicity. An excessive animal body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

An initial T/C ≥ 125% is considered necessary to demonstrate moderate activity. A reproducible T/C ≥ 150% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹Cancer Research 1 pp. 919-938, 1941, and Cancer Research 30, pp. 2394-2400, 1970.

*Positive control NSC 409962 is BCNU. CAS RN is 154-93-8.

(3LE31 or 3LE21) Intraperitoneally-Implanted L 1210 Leukemia

Origin of Tumor Line: Chemically induced in 1948 in the spleen and lymph nodes of a DBA mouse by painting the skin with methylcholanthrene in ethyl ether.¹

Summary of Test Procedures: 1×10^5 cells in ascitic fluid are implanted i.p. in CD_2F_1 or $B_6D_2F_1$ mice. I.p. test agent treatment starts one day after tumor implant and continues daily for a total of nine injections. The parameter is median or mean survival time. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation: DBA/2 mice ($B_6D_2F_1$ or CD_2F_1 for one generation if DBA/2 are not available).

Testing: CD_2F_1 (preferred) or $B_6D_2F_1$ mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Six animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Suspension: Prepare 0.1 ml of diluted ascitic fluid containing 1×10^5 cells.

Time: Day 6 or 7.

Site: Implant i.p. 0.1 ml of suspension.

TESTING

Suspension: Prepare 0.1 ml of diluted ascitic fluid containing 1×10^5 cells.

Time: Day 6 or 7.

Site: Implant i.p. 0.1 ml of suspension.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every odd-numbered experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize and weigh animals. Treat as instructed.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Toxicity day for test animals and second animal weigh day. Prepare fresh test agent for subsequent testing.

Day 6: Control early-death day.

Day 18: Control no-take day.

Day 20: If there are no survivors except for those treated with positive control compound, end and evaluate experiment.

Day 30: End and evaluate experiment. For special studies including schedule dependency, animals are held 60 days.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 19893* at a dose of 20 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. QD 1-9. The lower T/C limit for the positive control is 135%. The acceptable untreated control mean/median survival time is 8-11 days**.

Evaluation: (refer to Protocol 11)

The parameter measured is median or mean survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with $> 65\%$ survivors on Day 5. A T/C value of $< 86\%$ indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

An initial T/C $\geq 125\%$ is considered necessary to demonstrate moderate activity. A reproducible T/C $\geq 150\%$ is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹J. National Cancer Institute, 10 pp 179-192 1949, and J. National Cancer Institute, 13(5) p. 1328, 1953

*Positive control compound NSC 19893 is 5-FU CAS RN is 51-21-8
**Changed to 7.5-10.5 as of April 25, 1983

(3LE37 or 3LE27) Intracranially-Implanted L1210 Leukemia

Origin of Tumor Line: Chemically induced in 1948 in the spleen and lymph nodes of a DBA mouse by painting the skin with methylcholanthrene in ethyl ether.¹

Summary of Test Procedures: 1×10^4 cells in ascitic fluid are implanted i.c. in CD₂F₁ or B₆D₂F₁ mice. I.p. test agent treatment starts one day after tumor implant and continues daily for a total of nine injections. The parameter is median or mean survival time. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation: DBA/2 mice (B₆D₂F₁ or CD₂F₁ for one generation if DBA/2 are not available).

Testing: CD₂F₁ (preferred) or B₆D₂F₁ mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Suspension: Prepare 0.1 ml of diluted ascitic fluid containing 1×10^5 cells.

Time: Day 6 or 7.

Site: Implant i.p. 0.1 ml of suspension.

TESTING

Suspension: Prepare 0.05 ml of diluted ascitic fluid containing 1×10^4 cells.

Time: Day 6 or 7.

Site: Implant i.c. 0.05 ml of suspension.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every odd-numbered experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize and weigh animals. Treat as instructed.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Toxicity day for test animals and second animal weigh day. Prepare fresh test agent for subsequent testing.

Day 6: Control early-death day.

Day 21: Control no-take day.

Day 30: End and evaluate experiment. For special studies, animals are held 60 days.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 409962* at doses of 32 and 24 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. Day 1 only. The lower T/C limit for the positive control is 135%. The acceptable untreated control mean/median survival time is 8-11 days.

Evaluation: (refer to Protocol 11)

The parameter measured is median or mean survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with > 65% survivors on Day 5. A T/C value of < 86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

An initial T/C $\geq 125\%$ is considered necessary to demonstrate moderate activity. A reproducible T/C $\geq 150\%$ is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹J. National Cancer Institute, 10 pp 179-192, 1949, and J. National Cancer Institute, 13(5) p 1328, 1953.

*Positive control compound NSC 409962 is BCNU. CAS RN is 154-93-8

(3LKG5) Subrenal Capsule Human Lung LX-1 Xenograft

Origin of Tumor Line: Surgical explant in April, 1975 from a subcutaneous metastatic tumor of the right arm of a 48 year old man with oat cell lung carcinoma who had been treated in February, 1975 with C. Parvum, cyclophosphamide, and radiation. Carried in athymic mice, the tumor consists of a poorly differentiated carcinoma with no evidence of gland formation or mucin production. (Reference: Tumor Bank Information.)

Summary of Test Procedures: A tumor fragment is implanted under the membranous covering of the kidney of either athymic Swiss or athymic random bred mice. S.c.* test agent treatment starts one day after tumor implant and is repeated every fourth day for a total of three injections. The parameter is change in tumor weight.

Animals: (refer to Protocol 8)

Propagation and Testing: Athymic Swiss (Cr:NIH(S)-nu) or athymic random bred (NCR-nu) mice.

Weight: Mice should be within a 4 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Age: Record age of mice.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size:

General Testing: Six animals per test group and 12 per control. Typically, a single test agent experiment is run with 4 dose levels of the test agent at 50% intervals, using 6 mice per test group and 12 mice per vehicle control group. Total number of mice in a typical single test agent experiment (tests and control) is 36.

Tumor Transfer: (refer to Protocols 2, 5, and 6)

PROPAGATION

Fragment: Prepare a 2×2×2 mm fragment of s.c. donor tumor.

Time: When donor tumor reaches 500-800 mg (approximately Day 14).

Site: Implant fragment s.c. into axillary region with puncture in inguinal region using a 13 gauge trocar.

TESTING

Fragment: Prepare a 10×10×10 Ocular Micrometer Unit (OMU) fragment. Average diameter must be 9-12 OMU's measured under a dissecting microscope. 10 OMU's = 1 mm.

Anesthetic: Any satisfactory anesthetic (e.g., chloral hydrate, Avertin, etc.).

Medium: Tissue culture medium with no antibiotics (e.g., 199, Eagles MEM, or Earles).

Time: When donor tumor reaches 500-800 mg (approximately Day 14).

Site: Implant fragment under the subrenal capsule, using a 16 gauge trocar with a 22° bevel, after exposing the kidney with a 7 mm dorsal skin incision. The wound is closed with a 9 mm wound clip after closing the peritoneum with 1-4 silk sutures.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Anesthetize animals. Record body weight (Weigh Day 1). Implant tumor, measure, and record. Randomize animals after they recover from the anesthetic. Run bacterial cultures (refer to Protocol 7). Determine solubilities of test agents. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Prepare materials. Initiate s.c.* test agent injections (in the nape of the neck) based on individual body weight. Treatment is Q4D on Days 1, 5, and 9. Prepare test agent fresh on each injection day and administer based on individual body weight for that day.

Day 2: Recheck cultures. Discard experiment if contaminated, and report accordingly.

Days 5 and 9: Prepare test agent fresh on each injection day and administer based on individual body weight for that day.

Day 11: End and evaluate experiment. Record body weights (Weigh Day 2). Measure tumor in OMU's and record. (Final Evaluation Day for this model is also test toxicity day, test no-take day, control early-death day, control no-take day, and Weigh Day 2.)

Quality Control:

- 1) Quality control of this tumor line is the responsibility of the Tumor Bank. Tumors not properly sensitive to a positive control test agent will not be supplied.
- 2) Implant 2 or 3 additional mice which can be used for replacement for surgical deaths. If surgical deaths do not occur, use these mice as additional control animals.
- 3) Within a given experiment, whenever possible use mice from the same supplier, date of receipt, and shipping crate to reduce fighting. If mice fight, house fighters individually.
- 4) House mice 3 to 6 per cage.
- 5) Donor tumor should weigh between 500-800 mg and be scrupulously cleaned of necrotic and/or hemorrhagic areas.
- 6) In case of unusual deaths, these animals should be autopsied and peculiarities noted.
- 7) Specific definitions for subrenal capsule implants for Control Status Code assignments by the computer (refer to Protocol 7.7) are:
 - a) Acceptable control mean tumor weight change is $\geq 20\%$ between Day 0 and Final Evaluation Day.
 - b) Control no-take: A mouse with a tumor weight increase of $< 20\%$ between Day 0 and Final Evaluation Day. (Computer determined.)
 - c) Excessive control no-takes: 2 or more no-takes are excessive in a control group of 12 mice (refer to Protocol 7.3).
 - d) Excessive control early-deaths: 2 or more control deaths in a group of 12 to 19 animals (i.e., $\geq 10\%$) on or before Final Evaluation Day.

Evaluation: (refer to Protocol 11)

The parameter measured is mean tumor weight change (delta) based on length and width measurements in millimeters. Compute mean animal body weights for Day 1 and Day 11, compute T/C for all test groups with > 65% survivors on Day 11. An excessive animal body weight change difference (test minus control) may also be used in evaluating toxicity.

The NCI screening laboratories on Day 0 and on Final Evaluation Day are to measure and input OMU length and width measurements for tumors. The dimensions are measured and recorded in Ocular Micrometer Units (OMU). (They will be entered on the WS 180 Solid Tumor Data Form using *type 2* with *code H*, per instructions of 9/81 from the Screener Instructions for Use of the Solid Tumor Input Form, section 3.3.1.2. By convention, the length (L) dimension must be entered first.) The NCI computer:

- 1) Converts OMU's to millimeters (mm).
- 2) Calculates tumor weights (mgs) from tumor dimensions (mm × mm) following the formula for the volume of a prolate ellipsoid:

$$\frac{L \times W^2}{2} \quad \text{Where L is the longer of the two measurements.}$$

- 3) Calculates the change (delta) in mean tumor weight for each group of mice:

$$\text{Change in Mean Tumor Weight} = \text{Mean Tumor Weight}_{\text{FINAL}} - \text{Mean Tumor Weight}_{\text{INITIAL}}$$

- 4) Calculates the change (delta) in mean tumor weight for test (T) and control (C) groups.

- 5) Calculates T/C% for all test groups with > 65% survivors on Final Evaluation Day:

$$T/C\% = \frac{\Delta WtT}{\Delta WtC} \times 100 \text{—if } \Delta WtT \text{ positive.}$$

$$T/C\% = \frac{\Delta WtT}{\text{Test Mean Tumor Weight}_{\text{INITIAL}}} \times 100 \text{—if } \Delta WtT \text{ negative.}$$

Criteria for Activity:

An initial T/C ≤ 20% is considered necessary to demonstrate moderate activity. A reproducible T/C ≤ 10% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports. Input data. Screener assigns a code of "U" to an individual mouse whose response screener considers invalid, including the following circumstances:

- 1) Tumor lost from site of implant and kidney appears normal.
- 2) Animal dies or appears ill and loses weight—not attributable to test agent toxicity—for any reason, including fighting.
- 3) More than 1 tumor present.
- 4) Infection at site of implant.
- 5) Kidney does not appear normal.

A comment must accompany all "U" code designations.

The screener designates as unsatisfactory (assigns a Test Status Code of 33) all test groups that the screener considers invalid for any reason, including a case where more than 33% of the mice have been assigned a "U" code. The computer designates as unsatisfactory (assigns a Test Status Code of 34) all tests where:

- 1) There is no control delta calculated.
- 2) More than 33% of test mice have been assigned a "U" code.
- 3) Less than 67% of "Non-U" test mice are acceptable for calculation (i.e., initial tumor diameters are between 9 to 12 OMU's and final measurements exist for Final Evaluation Day).
- 4) Test groups where the control group contains 10% or more spontaneous tumor regressions.

The computer assigns the appropriate Control Status Code to reflect the acceptability of the control group, using definitions listed under "7" of Quality Control, in addition to the general definitions of Protocol 7.7 and Instruction 1.4.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

(3LKH2) Subcutaneously-Implanted Human Lung LX-1 Xenograft

Origin of Tumor Line: Surgical explant in April, 1975 from a subcutaneous metastatic tumor of the right arm of a 48 year old man with oat cell lung carcinoma who had been treated in February, 1975 with C. Parvum, cyclophosphamide, and radiation. Carried in athymic mice, the tumor consists of a poorly differentiated carcinoma with no evidence of gland formation or mucin production. (Reference: Tumor Bank information.)

Summary of Test Procedures: A tumor fragment is implanted subcutaneously in the axillary region of either athymic Swiss or athymic random bred mice. I.p. test agent treatment starts when tumors reach a weight range of 100-700 mg and is repeated every fourth day for a total of three injections. The parameter is change in tumor weight.

Animals: (refer to Protocol 8)

Propagation and Testing: Athymic Swiss (Cr:NIH(S)-nu) or athymic random bred (NCR-nu) mice.

Weight: Mice should be within a 4 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Age: Record age of mice.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Six to 10 earmarked animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)

PROPAGATION

Fragment: Prepare a 25 mg fragment of s.c. donor tumor.

Time: Day 28 (approximately).

Site: Implant 25 mg fragment s.c. into the axillary region with a puncture in the inguinal region.

TESTING

Fragment: Prepare a 14 mg fragment of s.c. donor tumor.

Time: Day 28 (approximately).

Site: Implant a 14 mg fragment s.c. into the axillary region with a puncture in the inguinal region.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7).

Day 1: Check cultures. Discard experiment if contaminated. Record deaths daily.

Day 2: Recheck cultures. Discard experiment if contaminated.

Staging Day (Initial Treatment Day): Select mice with tumors weighing no less than 100 mg and no more than 700 mg.

Prepare materials. Randomize and treat by individual body weight. Inject test agent i.p. on Staging Day and continue every 4 days for a total of 3 injections. Record total group body weight (Weigh Day 1).

Measurement Days: Body weights and tumor measurements are recorded on Initial Treatment Day (Staging Day), and selected measurement days.

Final Evaluation Day: Variable—that measurement day which yields the optimum (best) T/C% is designated Final Evaluation Day. End and evaluate experiment.

Quality Control: (refer to Protocol 7)

Not established.

Evaluation: (refer to Protocol 11)

The parameter measured is mean tumor weight change (delta) based on length and width measurements in millimeters.

Tumors are considered eligible for evaluation in the period 7 to 21 days post initial treatment for single injection treatment and 12 to 21 days post initial treatment for all other regimens. Measurement days should be selected such that the entire evaluation eligibility period is examined (i.e., post initial treatment days 13, 17, and 21 as an example). T/C% is calculated for only those groups where the survivors on the final possible evaluation day are greater than 65%. An excessive animal body weight change difference (test minus control) may also be used in evaluating toxicity.

Parameter: Calculations are based on group weights. Animals are earmarked only to permit total elimination of data for any animal designated as a no-take, any animal that escaped, or any animal that for any reason is deemed by the screener to be unacceptable for inclusion in the calculations.

Mean tumor weights are calculated for each measurement day within the eligibility period as each day is potentially a Final Evaluation Day. For each such day, change (delta) in mean tumor weight is calculated for both the test and control, as follows:

1) Calculate the change (delta) in mean tumor weight for test (T) and control (C) groups of mice:

Change (delta) in Mean Tumor Weight (Δ Wt) =

Mean Tumor Weight_{FINAL} - Mean Tumor Weight_{INITIAL}.

2) Calculate T/C% for all test groups with greater than 65% survivors on post staging Day 21.

$$T/C\% = \frac{\Delta WtT}{\Delta WtC} \times 100 \text{ — if } \Delta WtT \text{ positive.}$$

$$T/C\% = \frac{\Delta WtT}{\text{Test Mean Tumor Weight}_{\text{INITIAL}}} \times 100 \text{ — if } \Delta WtT \text{ negative.}$$

Determine optimum T/C% and the day of its occurrence to establish the final T/C% evaluation and Final Evaluation Day.

Criteria for Activity:

An initial T/C \leq 20% is considered necessary to demonstrate moderate activity. A reproducible T/C \leq 10% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

(3LL32) Subcutaneously-Implanted Lewis Lung Carcinoma

Origin of Tumor Line: Arose spontaneously in 1951 as a carcinoma of the lung in a C57BL/6 mouse.¹

Summary of Test Procedures: A tumor fragment is implanted subcutaneously in the axillary region of a B₆D₂F₁ mouse. I.p. test agent treatment starts one day after tumor implant and continues daily for a total of nine injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation: C57BL/6 mice.

Testing: B₆D₂F₁ mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups with a minimum of 40 control animals.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Fragment: Prepare a 2-4 mm fragment of s.c. donor tumor.

Time: Day 13-15.

Site: Implant the fragment s.c. in the axillary region with a puncture in the inguinal region.

TESTING

Fragment: Prepare a 2-4 mm fragment of s.c. donor tumor.

Time: Day 13-15.

Site: Implant the fragment s.c. in the axillary region with a puncture in the inguinal region.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Test positive control compound in every odd-numbered experiment. Prepare materials. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize animals. Treat as instructed. Administer test agent based on individual body weight.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Weigh Day 2 and day of initial test agent toxicity evaluation.

Day 14: Control early-death day.

Day 48: Control no-take day.

Day 60: End and evaluate experiment. Examine lungs grossly for tumor.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 26271* at a dose of 100 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. Day 1 only. The lower T/C limit for the positive control is 140%. The acceptable untreated control median survival time is 19-35.6 days.

Evaluation: (refer to Protocol 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with > 65% survivors on Day 5. A T/C value < 86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

An initial T/C \geq 140% is considered necessary to demonstrate moderate activity. A reproducible T/C value \geq 150% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹Cancer Research, 15: pp 38-51, 1955.

*Positive control compound NSC 26271 is Cytosin CAS RN is 50-18-0

(3LL39) Intravenously-Implanted Lewis Lung Carcinoma

Origin of Tumor Line: Arose spontaneously in 1951 as a carcinoma of the lung in a C57BL/6 mouse ¹

Summary of Test Procedures: A 1×10^6 cellular suspension of tumor cells is inoculated via the tail vein. I.p. test agent treatment starts one day after tumor implant and continues daily for a total of nine injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation. C57BL/6 mice.

Testing: B₆C₃F₁ (preferred) or B₆D₂F₁ mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups with a minimum of 40 control animals.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Suspension: Prepare a suspension of 1×10^7 total count of intact tumor cells of s.c. donor tumors (a minimum of 3 mice).

Add meticulously selected non-necrotic tumor tissue from a minimum of 3 tumors to a hand tissue homogenizer adding in the process a small amount of cold balanced salt solution. Decant into a second homogenizer and repeat to remove cell clumps.

Time: Day 13-15.

Site: Implant s.c. into the axillary region with a puncture in the inguinal region, a 0.5 ml suspension containing 1×10^7 cells.

TESTING

Suspension: Prepare a suspension of 1×10^6 total count of intact tumor cells of s.c. donor tumors (a minimum of 3 mice).

Add meticulously selected non-necrotic tumor tissue from a minimum of 3 tumors to a hand tissue homogenizer adding in the process a small amount of cold balanced salt solution. Decant into a second homogenizer and repeat to remove cell clumps. (Tumor material must be kept on ice.)

Time: Day 13-15.

Site: Inoculate into the tail vein, using a 26 or 27 gauge needle, a 0.2 ml suspension containing 1×10^6 cells. Mice should be huddled approximately 75 per holding cage prior to injection and the tail wiped with alcohol to dilate the tail vein. Animals must be discarded if the plunger is pushed into the syringe in an unsuccessful attempt to inoculate via the tail vein.

Introduction of the New Tumor Bank Lines: A new tumor line received from the tumor bank (generation 1) will be passed for two additional generations before use on test. If generation 3 is not acceptable for test use, request a replacement from the tumor bank.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Randomize animals. Test positive control compound in every odd-numbered experiment. Prepare materials. Record deaths daily.

Day 1: Check cultures. Discard experiments if contaminated. Administer test agent based on individual body weight.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Weigh/Day 2 and day of initial test agent toxicity evaluation.

Day 13: Control early-death day.

Day 42: Control no-take day.

Day 60: End and evaluate experiment. Examine lungs grossly for tumor.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 26271* at doses of 120 and 90 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. Day 1 only. The lower T/C limit for the positive control is 140%. The acceptable untreated control median survival time is 16-26 days.

Evaluation: (refer to Protocol 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with > 65% survivors on Day 5. A T/C value < 86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

An initial T/C $\geq 140\%$ is considered necessary to demonstrate moderate activity. A reproducible T/C value $\geq 150\%$ is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹Cancer Research, 15 pp. 38-55, 1955

*Positive control compound NSC 26271 is Cytosar CAS RN is 50-18-0

(3MBG5) Subrenal Capsule Human Mammary Carcinoma MX-1 Xenograft

Origin of Tumor Line: Surgical explant in 1974 from the primary mammary tumor of a 29 year old woman with no previous chemotherapy. Carried in athymic mice, the tumor is a poorly differentiated mammary carcinoma, highly cellular with no evidence of gland formation or mucin production. (Reference: Tumor Bank information.)

Summary of Test Procedures: A tumor fragment is implanted under the membranous covering of the kidney of either athymic Swiss or athymic random bred mice. S.c.* test agent treatment starts one day after tumor implant and is repeated every fourth day for a total of three injections. The parameter is change in tumor weight.

Animals: (refer to Protocol 8)

Propagation and Testing: Athymic Swiss (Cr:NIH(S)-nu) or athymic random bred (NCR-nu) mice.

Weight: Mice should be within a 4 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Age: Record age of mice.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size:

General Testing: Six animals per test group and 12 per control. Typically, a single test agent experiment is run with 4 dose levels of the test agent at 50% intervals, using 6 mice per test group and 12 mice per vehicle control group. Total number of mice in a typical single test agent experiment (tests and control) is 36.

Tumor Transfer: (refer to Protocols 2, 5, and 6)

PROPAGATION

Fragment: Prepare a 2X2X2 mm fragment of s.c. donor tumor.

Time: When donor tumor reaches 500-800 mg (approximately Day 14).

Site: Implant fragment s.c. into axillary region with puncture in inguinal region using a 13 gauge trocar.

TESTING

Fragment: Prepare a 10X10X10 Ocular Micrometer Unit (OMU) fragment. Average diameter must be 9-12 OMU's measured under a dissecting microscope. 10 OMU's = 1 mm.

Anesthetic: Any satisfactory anesthetic (e.g., chloral hydrate, Avertin, etc.).

Medium: Tissue culture medium with no antibiotics (e.g., 199, Eagles MEM, or Earles).

Time: When donor tumor reaches 500-800 mg (approximately Day 14).

Site: Implant fragment under the subrenal capsule, using a 16 gauge trocar with a 22° bevel, after exposing the kidney with a 7 mm dorsal skin incision. The wound is closed with a 9 mm wound clip after closing the peritoneum with 1-4 silk sutures.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Anesthetize animals. Record body weight (Weigh Day 1). Implant tumor, measure, and record. Randomize animals after they recover from the anesthetic. Run bacterial cultures (refer to Protocol 7). Determine solubilities of test agent. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Prepare test materials. Initiate s.c.* test agent injections (in the nape of the neck) based on individual body weight. Treatment is Q4D on Days 1, 5, and 9. Prepare test agent fresh on each injection day and administer based on individual body weight for that day.

Day 2: Recheck cultures. Discontinue testing if contaminated and report accordingly.

Days 5 and 9: Prepare test agent fresh on each injection day and administer based on individual body weight for that day.

Day 11: End and evaluate experiment. Record body weights (Weigh Day 2). Measure tumor in OMU's and record. Final Evaluation Day for this model is also test toxicity day, test no-take day, control early-death day, control no-take day, and Weigh Day 2.

Quality Control:

- 1) Quality control of this tumor line is the responsibility of the Tumor Bank. Tumors not properly sensitive to a positive control test agent will not be supplied.
- 2) Implant 2 or 3 additional mice which can be used for replacement for surgical deaths. If surgical deaths do not occur, use these mice as additional control animals.
- 3) Within a given experiment, whenever possible use mice from the same supplier, date of receipt, and shipping crate to reduce fighting. If mice fight, house fighters individually.
- 4) House mice 3 to 6 per cage.
- 5) Donor tumor should weigh between 500-800 mg and be scrupulously cleaned of necrotic and/or hemorrhagic areas.
- 6) In case of unusual deaths, these animals should be autopsied and peculiarities noted.
- 7) Specific definitions for subrenal capsule implants for Control Status Code assignments by the computer (refer to Protocol 7.7) are:
 - a) Acceptable control mean tumor weight change is $\geq 20\%$ between Day 0 and Final Evaluation Day.
 - b) Control no-take: A mouse with a tumor weight increase of $< 20\%$ between Day 0 and Final Evaluation Day. (Computer determined.)

- c) Excessive control no-takes: 2 or more no-takes are excessive in a control group of 12 mice (refer to Protocol 7.3).
- d) Excessive control early deaths: 2 or more control deaths in a group of 12 to 19 animals (i.e., $\geq 10\%$) on or before Final Evaluation Day.

Evaluation: (refer to Protocol 11)

The parameter measured is mean tumor weight change (delta) based on length and width measurements in millimeters. Compute mean animal body weights for Day 1 and Day 11, compute T/C for all test groups with $> 65\%$ survivors on Day 11. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

The NCI screening laboratories on Day 0 and on Final Evaluation Day are to measure and input OMU length and width measurements for tumors. The dimensions are measured and recorded in Ocular Micrometer Units (OMU). (They will be entered on the WS 180 Solid Tumor Data Form using type 2 with code H, per instructions of 9/81 from the Screener Instructions for Use of the Solid Tumor Input Form, section 3.3.1.2. By convention, the length (L) dimension must be entered first). The NCI computer:

- 1) Converts OMU's to millimeters (mm).
- 2) Calculates tumor weights (mgs) from tumor dimensions (mm \times mm) following the formula for the volume of a prolate ellipsoid:

$$\frac{L \times W^2}{2} \quad \text{Where L is the longer of the two measurements.}$$

- 3) Calculates the change (delta) in mean tumor weight for each group of mice:

$$\text{Change in Mean Tumor Weight} = \text{Mean Tumor Weight}_{\text{FINAL}} - \text{Mean Tumor Weight}_{\text{INITIAL}}$$

- 4) Calculates the change (delta) in mean tumor weight for test (T) and control (C) groups.

- 5) Calculates T/C% for all test groups with $> 65\%$ survivors on Final Evaluation Day:

$$T/C\% = \frac{\Delta WtT}{\Delta WtC} \times 100 \text{ — if } \Delta WtT \text{ positive.}$$

$$T/C\% = \frac{\Delta WtT}{\text{Test Mean Tumor Weight}_{\text{INITIAL}}} \times 100 \text{ — if } \Delta WtT \text{ negative.}$$

Criteria for Activity:

An initial T/C $\leq 20\%$ is considered necessary to demonstrate moderate activity. A reproducible T/C $\leq 10\%$ is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports. Input data. Screener assigns a code of "U" to an individual mouse whose response screener considers invalid, including the following circumstances:

- 1) Tumor lost from site of implant and kidney appears normal.
- 2) Animal dies or appears ill and loses weight—not attributable to test agent toxicity—for any reason, including fighting.
- 3) More than 1 tumor present.
- 4) Infection at site of implant.
- 5) Kidney does not appear normal.

A comment must accompany all "U" code designations.

The screener designates as unsatisfactory (assigns a Test Status Code of 33) all test groups that the screener considers invalid for any reason, including a case where more than 33% of the mice have been assigned a "U" code. The computer designates as unsatisfactory (assigns a Test Status Code of 34) all tests where:

- 1) There is no control delta calculated.
- 2) More than 33% of test mice have been assigned a "U" code.
- 3) Less than 67% of "Non-U" test mice are acceptable for calculation (i.e., initial tumor diameters are between 9 to 12 OMU's and final measurements exist for Final Evaluation Day).
- 4) Test groups where the control group contains 10% or more spontaneous tumor regressions.

The computer assigns the appropriate Control Status Code to reflect the acceptability of the control group, using definitions listed under "7" of Quality Control in addition to the general definitions of Protocol 7.7 and Instruction 14.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

(3MBH2) Subcutaneously-Implanted Human Mammary Carcinoma MX-1 Xenograft

Origin of Tumor Line: Surgical explant in 1974 from the primary mammary tumor of a 29 year old woman with no previous chemotherapy. Carried in athymic mice, the tumor is a poorly differentiated mammary carcinoma, highly cellular with no evidence of gland formation or mucin production. (Reference: Tumor Bank information.)

Summary of Test Procedures: A tumor fragment is implanted subcutaneously in the axillary region of either athymic Swiss or athymic random bred mice. I.p. test agent treatment starts when the tumors reach a weight range of 100-700 mg and is repeated every fourth day for a total of three injections. The parameter is change in tumor weight.

Animals: (refer to Protocol 8)

Propagation and Testing: Athymic Swiss (Cr:NIH(S)-nu) or athymic random bred (NCR-nu) mice.

Weight: Mice should be within a 4 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Age: Record age of mice.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Six to 10 earmarked animals per test group.

Control Group: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)

PROPAGATION

Fragment: Prepare a 25 mg fragment of s.c. donor tumor.

Time: Day 28 (approximately).

Site: Implant fragment s.c. into the axillary region with a puncture in the inguinal region.

TESTING

Fragment: Prepare a 14 mg fragment of s.c. donor tumor.

Time: Day 28 (approximately).

Site: Implant fragment s.c. into the axillary region with a puncture in the inguinal region.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7).

Day 1: Check cultures. Discard experiment if contaminated. Record deaths daily.

Day 2: Recheck cultures. Discard experiment if contaminated.

Staging Day (Initial Treatment Day): Select mice with tumors weighing no less than 100 mg and no more than 700 mg.

Prepare materials. Randomize and treat by individual body weight. Inject test agent i.p. on Staging Day and continue every 4 days for a total of 3 injections. Record total group body weight (Weigh Day 1).

Measurement Days: Body weights and tumor measurements are recorded on Initial Treatment Day (Staging Day) and selected measurement days.

Final Evaluation Day: Variable—that measurement day which yields the optimum (best) T/C% is designated Final Evaluation Day. End and evaluate experiment.

Quality Control: (refer to Protocol 7)

Not established.

Evaluation: (refer to Protocol 11)

The parameter measured is mean tumor weight change (delta) based on length and width measurements in millimeters.

Tumors are considered eligible for evaluation in the period 7 to 21 days post initial treatment for single injection treatment and 12 to 21 days post initial treatment for all other regimens. Measurement days should be selected such that the entire evaluation eligibility period is examined (i.e., post initial treatment days 13, 17, and 21 as an example). T/C% is calculated for only those groups where the survivors on the final possible evaluation day are greater than 65%. An excessive animal body weight change difference (test minus control) may also be used in evaluating toxicity.

Parameter: Calculations are based on group weights. Animals are earmarked only to permit total elimination of data for any animal designated as a no-take, any animal that escaped, or any animal that for any reason is deemed by the screener to be unacceptable for inclusion in the calculations.

Mean tumor weights are calculated for each measurement day within the eligibility period as each day is potentially a Final Evaluation Day. For each such day, change (delta) in mean tumor weight is calculated for both the test and control, as follows:

1) Calculate the change (delta) in mean tumor weight for test (T) and control (C) groups of mice:

Change (delta) in Mean Tumor Weight (Δ Wt) =

Mean Tumor Weight_{FINAL} - Mean Tumor Weight_{INITIAL}.

2) Calculate T/C% for all test groups with greater than 65% survivors on post staging Day 21.

$$T/C\% = \frac{\Delta WtT}{\Delta WtC} \times 100 \text{ — if } \Delta WtT \text{ positive.}$$

$$T/C\% = \frac{\Delta WtT}{\text{Test Mean Tumor Weight}_{\text{INITIAL}}} \times 100 \text{ — if } \Delta WtT \text{ negative.}$$

Determine optimum T/C% and the day of its occurrence to establish the final T/C% evaluation and Final Evaluation Day.

Criteria for Activity:

An initial T/C \leq 20% is considered necessary to demonstrate moderate activity. A reproducible T/C \leq 10% is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

(3PS31) Intraperitoneally-Implanted P388 Leukemia

Origin of Tumor Line: Chemically induced in 1955 in a DBA/2 mouse by painting the skin with 3-methylcholanthrene.¹

Summary of Test Procedures: 1×10^6 cells in ascitic fluid are implanted i.p. in CD₂F₁ mice. I.p. test agent treatment starts one day after tumor implant and is continued daily for a total of five injections for Synthetics, and a total of nine injections for crude Natural Products. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation: DBA/2 mice (B₆D₂F₁ or CD₂F₁ for one generation if DBA/2 are not available).

Testing: CD₂F₁ mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Six animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Suspension: Prepare a suspension of diluted ascitic fluid so that 0.1 ml portion contains 1×10^6 cells.

Time: Day 7.

Site: Implant i.p. 0.1 ml of suspension containing 1×10^6 cells.

TESTING

Suspension: Prepare a suspension of diluted ascitic fluid so that 0.1 ml portion contains 1×10^6 cells.

Time: Day 7.

Site: Implant i.p. 0.1 ml of suspension containing 1×10^6 cells.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every odd-numbered experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Weight and randomize animals. Treat as instructed.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Weigh animals and record. Toxicity day.

Day 7: Control early-death day.

Day 18: Control no-take day.

Day 20: If there are no survivors except those treated with positive control compound, end and evaluate experiment.

Day 30: End and evaluate experiment.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 19893* at a dose of 20 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. QD 1-5 for Synthetics or i.p. QD 1-9 for crude Natural Products. The lower T/C limit for the positive control is 135%. The acceptable untreated control median survival time is 9-13 days.

Evaluation: (refer to Protocol 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with > 65% survivors on Day 5. A T/C value of < 86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

An initial T/C $\geq 120\%$ for Synthetics and $\geq 130\%$ for crude Natural Products is considered necessary to demonstrate moderate activity. A reproducible T/C $\geq 175\%$ is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹American Journal of Pathology, 33: No. 3, p 603, 1957

*Positive control compound NSC 19893 is 5-FU. CAS RN is 51-21-8

(3PS37) Intracranially-Implanted P388 Leukemia

Origin of Tumor Line: Chemically induced in 1955 in a DBA/2 mouse by painting the skin with 3-methylcholanthrene.¹

Summary of Test Procedures: 1×10^5 cells in ascitic fluid are implanted i.c. in CD_2F_1 mice. I.p. test agent treatment starts one day after tumor implant and continues daily for a total of nine injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

Animals: (refer to Protocol 8)

Propagation: DBA/2 mice ($B_6D_2F_1$ or CD_2F_1 for one generation if DBA/2 are not available).

Testing: CD_2F_1 mice.

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted as comments.

Experiment Size: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: Number of control animals varies according to number of test groups.

Tumor Transfer: (refer to Protocols 2, 5, and 6)**PROPAGATION**

Suspension: Prepare a suspension of diluted ascitic fluid so that 0.1 ml portion contains 1×10^6 cells.

Time: Day 7.

Site: Implant i.p. 0.1 ml of suspension containing 1×10^6 cells.

TESTING

Suspension: Prepare a suspension of diluted ascitic fluid so that 0.05 ml portion contains 1×10^5 cells.

Time: Day 6 or 7.

Site: Implant i.c. 0.05 ml of diluted ascitic fluid containing 1×10^5 cells.

Testing Schedule: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every odd-numbered experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Weigh and randomize animals. Treat as instructed.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Weigh animals and record. Toxicity day.

Day 7: Control early-death day.

Day 22: Control no-take day.

Day 30: End and evaluate experiment. If any test, other than the positive control compound, has more than half of its animals surviving on that day, postpone Final Evaluation Day until Day 60.

Quality Control: (refer to Protocol 7)

Schedule the positive control compound (NSC 409962* at doses of 32 and 24 mg/kg/injection) in every odd-numbered experiment, the regimen for which is i.p. Day 1 only. The lower T/C limit for the positive control is 135%. The acceptable untreated control median survival time is 9-13 days.

Evaluation: (refer to Protocol 11)

The parameter measured is median survival time. Compute mean animal body weights for Day 1 and Day 5, compute T/C for all test groups with $> 65\%$ survivors on Day 5. A T/C value of $< 86\%$ indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

Criteria for Activity:

An initial T/C $\geq 125\%$ is considered necessary to demonstrate moderate activity. A reproducible T/C $\geq 175\%$ is considered significant activity.

Reporting of Data:

On the final day of testing, prepare final control and test reports.

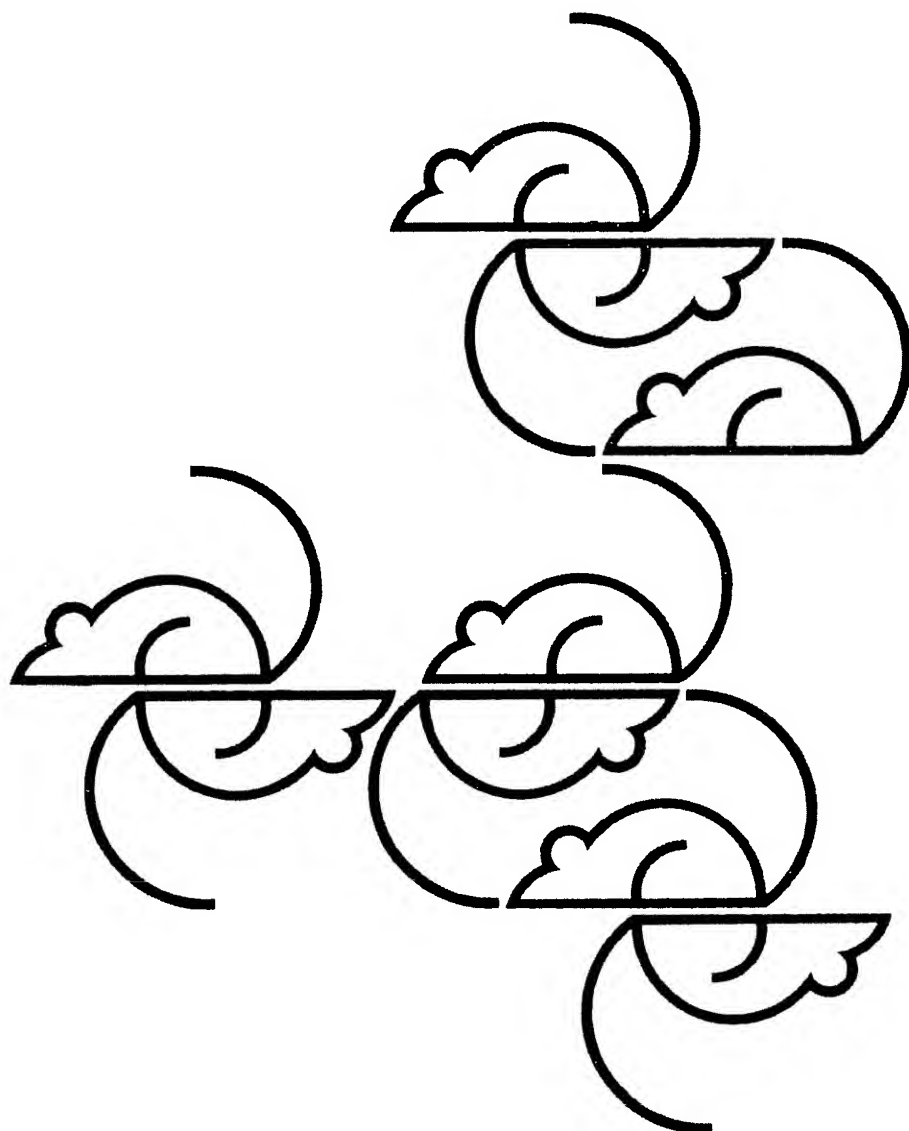
Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

¹American Journal of Pathology, 33 No. 3, p 603, 1957

*Positive control compound NSC 409962 is BCNU CAS RN is 154-93-8

Appendices



Appendix A

National Cancer Institute (NCI) Principal *In Vivo* Cancer Models 1976–1982 and Activity Criteria for Drug Testing

Model	Drug Route and Schedule	Moderate Activity Response (1)	Good Activity Response (2)
A. In Vivo Prescreen:			
3PS31—P388 Leukemia (IP)	IP QD Days 1–5	≥ 120	≥ 175
B. Mouse Tumor Homografts:			
3B131—B16 Melanoma (IP)	IP QD Days 1–9	≥ 125	≥ 150
3CD72—CD8F1 Mammary Adenocarcinoma (SC)	IP Q7D 1, 8, 15, 22, and 29	≤ 42	≤ 10
3CDJ2—CD8F1 Mammary Adenocarcinoma (SC)	IP Single Treatment on Staging Day	≤ 20	≤ 0
3C872—Colon 38 Carcinoma (SC)	IP Q7D Days 2 and 9	≤ 42	≤ 10
3LE31—L1210 Leukemia (IP)	IP QD Days 1–9	≥ 125	≥ 150
3LL39—Lewis Lung Carcinoma (IV)	IP QD Days 1–9	≥ 140	≥ 150
C. Human Tumor Xenografts Implanted Under Kidney Capsule of Mice:			
3C2G5—Colon CX-1 Adenocarcinoma	SC Q4D Days 1, 5, 9, and 13	≤ 20	≤ 10
3LKG5—Lung LX-1 Carcinoma	SC Q4D Days 1, 5, and 9	≤ 20	≤ 10
3MBG5—Mammary MX-1 Carcinoma	SC Q4D Days 1, 5, and 9	≤ 20	≤ 10
(1) = Confirmed response of test compared to control that permits consideration as tumor-panel candidate.			
(2) = Confirmed response of test compared to control that permits consideration for NCI development toward clinical trial.			

Appendix B

Instruction 271E-P

Summary of the usual characteristics of selected murine models used under the auspices of the NCI Division of Cancer Treatment (1,5,6)

Model	Tumor	Host		Approx Tumor Transfer Day	Propagation Inoculum			Test inoculum			Doubling Time (Days) ⁽⁴⁾	Quality Control ⁽¹²⁾	Mice Per Test
		Propagation	Testing		Site	Tissue ⁽¹⁾	Level Per Mouse	Site	Tissue ⁽¹⁾	Level Per Mouse			
3B131	B1—B16 melanoma	C57BL/6	B ₆ C ₃ F ₁ or B ₆ D ₂ F ₁ (BDF ₁)	12	SC	Fragment	25mg 1:10 Brei	IP	1:10 ⁽³⁾ Brei	0.5ml	1.3-2.8	2	10
3B132	B1—B16 melanoma	C57BL/6	B ₆ D ₂ F ₁ (BDF ₁)	12	SC	Fragment	25mg 1:10 Brei	SC	Fragment	25mg 1:10 Brei	(fragment) 0.8-1.6 NE	2	10
3B137	B1—B16 melanoma	C57BL/6	B ₆ D ₂ F ₁ (BDF ₁)	11	SC	Fragment	25mg 1:10 Brei	IC	1×10 ⁶ Cells ⁽³⁾	0.05ml	1.4	NE	10
3CDJ2	CD—CD8F1, mammary adenocarcinoma	CD8F1 ♀	CD8F1	NA		Spontaneous in CD8F1 Females		SC	1:20 Brei	0.3ml	1.6-4.1 ⁽¹⁰⁾	3	10
3CD72	CD—CD8F1, mammary adenocarcinoma	CD8F1 ♀	CD8F1	NA		Spontaneous in CD8F1 Females		SC	1:20 Brei	0.3ml	NE	3	10
3C2G5	C2—CX-1 colon adenocarcinoma xenograft	Cr:NIH(S)-nu or NCr-nu	Cr:NIH(S)-nu or NCr-nu	21	SC	Fragment	2×2×2mm	SRC ⁽⁷⁾	Fragment	1×1×1mm	5.0	NE	6
3C2H2	C2—CX-1 colon adenocarcinoma xenograft	Cr:NIH(S)-nu or NCr-nu	Cr:NIH(S)-nu or NCr-nu	35	SC	Fragment	25mg	SC	Fragment	14mg	8.6 ⁽¹¹⁾	NE	6-10
3C631	C6—colon 26 carcinoma	BALB/cJ	CD ₂ F ₁	13	SC	Fragment	100mg	IP	1:100 Brei	0.5ml	2.0-7.0	2.5	10
3C872	C8—colon 38 carcinoma	C57BL/6	B ₆ C ₃ F ₁ or B ₆ D ₂ F ₁ (BDF ₁)	20	SC	Fragment (13 Gauge Trocar)	70mg	SC	Fragment (13 Gauge Trocar)	70mg	0.7-3.2	3	10
3EM37 or 3EP37	EM or EP—Ependymoblastoma	C57BL/6	B ₆ C ₃ F ₁ or C57BL/6	14	SC	Fragment	2×2×8mm	IC	1×10 ⁶ Cells ⁽³⁾ Fragment	0.05ml 1×1×1mm	NE	2.75	10
3LE21 or 3LE31	LE—L-1210 leukemia	DBA/2	CD ₂ F ₁ or B ₆ D ₂ F ₁ (BDF ₁)	7	IP	1×10 ⁶ Cells	0.1ml	IP	1×10 ⁶ Cells ⁽³⁾	0.1ml	0.24-0.34	1	6
3LE27 or 3LE37	LE—L-1210 leukemia	DBA/2	CD ₂ F ₁ or B ₆ D ₂ F ₁ (BDF ₁)	7	IP	1×10 ⁶ Cells	0.1ml	IC	1×10 ⁶ Cells ⁽³⁾	0.05ml	0.4	2.75	10
3LK65	LK—LX-1 lung carcinoma xenograft	Cr:NIH(S)-nu or NCr-nu	Cr:NIH(S)-nu or NCr-nu	14	SC	Fragment	2×2×2mm	SRC ⁽⁷⁾	Fragment	1×1×1mm	2.2	NE	6
3LKH2	LK—LX-1 lung carcinoma xenograft	Cr:NIH(S)-nu or NCr-nu	Cr:NIH(S)-nu or NCr-nu	28	SC	Fragment	25mg	SC	Fragment	14mg	2.9 ⁽¹¹⁾	NE	6-10
3LL32	LL—Lewis lung carcinoma	C57BL/6	B ₆ D ₂ F ₁ (BDF ₁)	14	SC	Fragment	2-4mm	SC	Fragment	2-4mm	0.9-1.3	2	10
3LL39	LL—Lewis lung carcinoma	C57BL/6	B ₆ C ₃ F ₁ or B ₆ D ₂ F ₁ (BDF ₁)	14	SC	1×10 ⁶ Cells	0.5ml	IV	1×10 ⁶ Cells ⁽³⁾	0.2ml	0.9-2.1	2.75	10
3MBG5	MB—MX-1 mammary carcinoma xenograft	Cr:NIH(S)-nu or NCr-nu	Cr:NIH(S)-nu or NCr-nu	14	SC	Fragment	2×2×2mm	SRC ⁽⁷⁾	Fragment	1×1×1mm	2.6	NE	6
3MBH2	MB—MX-1 mammary carcinoma xenograft	Cr:NIH(S)-nu or NCr-nu	Cr:NIH(S)-nu or NCr-nu	28	SC	Fragment	25mg	SC	Fragment	14mg	3.9 ⁽¹¹⁾	NE	6-10
3PS31	PS—P388 leukemia	DBA/2	CD ₂ F ₁	7	IP	1×10 ⁶ Cells	0.1ml	IP	1×10 ⁶ Cells ⁽³⁾	0.1ml	0.39-0.52	1	6 (SYN) 4 (NP fraction except 6 for K fraction)
3PS37	PS—P388 leukemia	DBA/2	CD ₂ F ₁	7	IP	1×10 ⁶ Cells	0.1ml	IC	1×10 ⁶ Cells ⁽³⁾	0.05ml	0.6	NE	10

(1) Codes for data may be found in Instruction 14

(2) Treatment starts when tumors reach weight of 100 mg to 700 mg

(3) All cell counts are total intact cells

(4) Animal weight change-difference cutoff: Excess loss indicates possible toxicity; or possible false MC1 level/activity in a tumor-weight-inhibition test

(5) The information on the most recent revision of this chart takes precedence over previous instructions

(6) The next revision of this chart is in preparation—send your suggestions to your NCI project officer.

(7) SRC = Subrenal Capsule

(8) Brei dilution is grams of tumor tissue in milliliters of final Brei (e.g., in a 1:10 Brei, 10ml of Brei includes 1 gram of tumor tissue)

(9) Approximate doubling time (days) of tumor at implant size assuming 100% viability

(10) Approximate doubling time range for tumors between 100mg and 700mg on staging day

(11) Approximate doubling time for a 100mg tumor, which is the approximate tumor size at beginning of treatment

(12) Quality Control is the responsibility of the Tumor Bank

Drug Route & Schedule	Second Weigh Day	Test Toxicity Day	Control		Final Evaluation Day (FED)	Parameter (Relative to Control)	Acceptable Control Range	Positive Control Compound				Test T/C%				Model
			Early Death Day	No Take Day				NSC	Route & Treatment	Dose (mg/kg/ injection)	T/C%	Toxicity	Grams Negative ^a Body Weight Change Difference (T-C)	Activity Criterion	DN2 - Criterion (if Confirmed)	
IP QD D1-9	5	5	12	30	60	Median Survival Time	15-22 Days	119875	IP QD D1-9	2 & 1	≥ 135	≤ 85	≥ 4	≥ 125	≥ 150	3B131
IP QD D1-9	5	5	13	46	60	Median Survival Time	21-31 Days	19893 26271 125066	IP QD D1-9 IP QD D1-9 IP QD D1-9	20 50 12, 6 & 3	≥ 135 ≥ 135 ≥ 135	≤ 85	≥ 4	≥ 140	≥ 150	3B132
IP QD D1-9	5	5	7	24	30/60	Median Survival Time	11-18 Days	409962	IP Single-D1	32 & 24	≥ 140	≤ 85	≥ 4	≥ 125	≥ 150	3B137
IP Single On Staging Day ⁽²⁾	FED	FED	FED	FED	7 Days Post Staging Day	Median Tumor Weight Change	400-2000mg	26271	IP Single On Staging Day ⁽²⁾	250	≤ 20	NA	≥ 7	≤ 20	≤ 0	3CDJ2
IP Q7D×5 Starting Day 1	30	FED	FED	FED	30	Median Tumor Weight	300-5000mg	26271	IP Q7D×5 Starting Day 1	37.5	≤ 42	NA	NE	≤ 42	≤ 10	3CD72
SC Q4D×4 Starting Day 1	15	15	15	15	15	Change in Tumor Mass	≥ 20% Tumor Growth	(*) NA				NA	≥ 8	≤ 20	≤ 10	3C2G5
IP Q4D×3 ⁽²⁾	NE	NE	NE	NA	Variable	Change in Tumor Mass	NA	NE				NA	NE	≤ 20	≤ 10	3C2H2
IP Q4D×2 Starting Day 1	5	5	8	60	60	Median Survival Time	16-29 Days	95441	IP Q4D D1,5	10	≥ 150	≤ 85	≥ 4	≥ 130	≥ 150	3C631
IP Q7D×2 Starting Day 2	20	20	20	20	20	Median Tumor Weight	400-2000mg	19893	IP Q7D D2,9	70	≤ 42	NA	NA	≤ 42	≤ 10	3C872
IP QD D1-5	5	5	10	40	60	Median Survival Time	14-30 Days	409962	IP QD D1-5	8, 4 & 2	≥ 135	≤ 85	≥ 4	≥ 125	≥ 150	3EM37 or 3EP37
IP QD D1-9	5	5	6	18	30/60	Mean/Median Survival Time	8-11 Days	19893	IP QD D1-9	20	≥ 135	≤ 85	≥ 4	≥ 125	≥ 150	3LE21 or 3LE31
IP QD D1-9	5	5	6	21	30/60	Mean/Median Survival Time	8-11 Days	409962	IP Single-D1	32 & 24	≥ 135	≤ 85	≥ 4	≥ 125	≥ 150	3LE27 or 3LE37
SC Q4D×3 Starting Day 1	11	11	11	11	11	Change in Tumor Mass	≥ 20% Tumor Growth	(*) NA				NA	NE	≤ 20	≤ 10	3LKG5
IP Q4D×3 ⁽²⁾	NE	NE	NE	NA	Variable	Change in Tumor Mass	NA	NE				NA	NE	≤ 20	≤ 10	3LKH2
IP QD D1-9	5	5	14	48	60	Median Survival Time	19-35.6 Days	26271	IP Single-D1	100	≥ 140	≤ 85	≥ 4	≥ 140	≥ 150	3LL32
IP QD D1-9	5	5	13	42	60	Median Survival Time	16-26 Days	26271	IP Single-D1	120 & 90	≥ 140	≤ 85	≥ 4	≥ 140	≥ 150	3LL39
SC Q4D×3 Starting Day 1	11	11	11	11	11	Change in Tumor Mass	≥ 20% Tumor Growth	(*) NA				NA	≥ 6	≤ 20	≤ 10	3MBG5
IP Q4D×3 ⁽²⁾	NE	NE	NE	NA	Variable	Change in Tumor Mass	NA	NE				NA	NE	≤ 20	≤ 10	3MBH2
IP QD D1-5(SYN)								19893	IP QD D1-5	20				≥ 120 (SYN)		
IP QD D1 9(NP)	5	5	7	18	30	Median Survival Time	9-13 Days	19893	IP QD D1-9	20	≥ 135	≤ 85	≥ 4	≥ 130 (NP)	≥ 175	3PS31
IP QD D1-9	5	5	7	22	30/60	Median Survival Time	9-13 Days	409962	IP Single-D1	32 & 24	≥ 135	≤ 85	≥ 4	≥ 125	≥ 175	3PS37

Instruction No. 14 can be requested from: Information Technology Branch,
Developmental Therapeutics Program, Division of
Cancer Treatment, National Cancer Institute,
Bethesda, Maryland 20205

Approx - Approximate
MC - Material Classification (See Instruction 14)
NA - Not Applicable
NE - Not Established

THIS PAGE BLANK (USPTO)